

## University of Groningen

### Insect sex determination

Verhulst, Eveline C.; van de Zande, Louis; Beukeboom, Leo W.

*Published in:*  
Current opinion in genetics & development

*DOI:*  
[10.1016/j.gde.2010.05.001](https://doi.org/10.1016/j.gde.2010.05.001)

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2010

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Verhulst, E. C., van de Zande, L., & Beukeboom, L. W. (2010). Insect sex determination: It all evolves around transformer. *Current opinion in genetics & development*, 20(4), 376-383.  
<https://doi.org/10.1016/j.gde.2010.05.001>

#### Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

#### Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

# Insect sex determination: it all evolves around *transformer*

Eveline C Verhulst, Louis van de Zande and Leo W Beukeboom

Insects exhibit a variety of sex determining mechanisms including male or female heterogamety and haplodiploidy. The primary signal that starts sex determination is processed by a cascade of genes ending with the conserved switch *doublesex* that controls sexual differentiation. *Transformer* is the *doublesex* splicing regulator and has been found in all examined insects, indicating its ancestral function as a sex-determining gene. Despite this conserved function, the variation in *transformer* nucleotide sequence, amino acid composition and protein structure can accommodate a multitude of upstream sex determining signals. *Transformer* regulation of *doublesex* and its taxonomic distribution indicate that the *doublesex-transformer* axis is conserved among all insects and that *transformer* is the key gene around which variation in sex determining mechanisms has evolved.

## Address

Evolutionary Genetics, Centre for Ecological and Evolutionary Studies,  
 University of Groningen, The Netherlands

Corresponding author: Verhulst, Eveline C ([E.C.Verhulst@rug.nl](mailto:E.C.Verhulst@rug.nl))

**Current Opinion in Genetics & Development** 2010, **20**:376–383

This review comes from a themed issue on  
 Developmental mechanisms, patterning and evolution  
 Edited by Claude Desplan and Margaret Buckingham

Available online 1st June 2010

0959-437X/\$ – see front matter

© 2010 Elsevier Ltd. All rights reserved.

DOI [10.1016/j.gde.2010.05.001](https://doi.org/10.1016/j.gde.2010.05.001)

## Introduction

Sexual development, one of the most important and widespread developmental processes, essentially entails one simple choice: becoming male or female. Although this suggests a common underlying genetic mechanism, an astoundingly diverse array of pathways regulates sex determination. Sanchez [1•] reviewed current knowledge of sex determining mechanisms with a focus on primary signals. In flies (Diptera) the gene *doublesex* (*dsx*) acts as a conserved major switch at the bottom of the sex-determining cascade [1•,2,3]. The part of the sex-determining cascade where the primary signal is transmitted to *dsx* has, until recently, received less attention. Data from Hymenoptera enabled comparison of sex determination mechanisms at a wider level within the insect class. This has directed focus towards *transformer* (*tra*) as a central player in the evolution of sex determination in insects. In this review, we describe how *tra* translates different

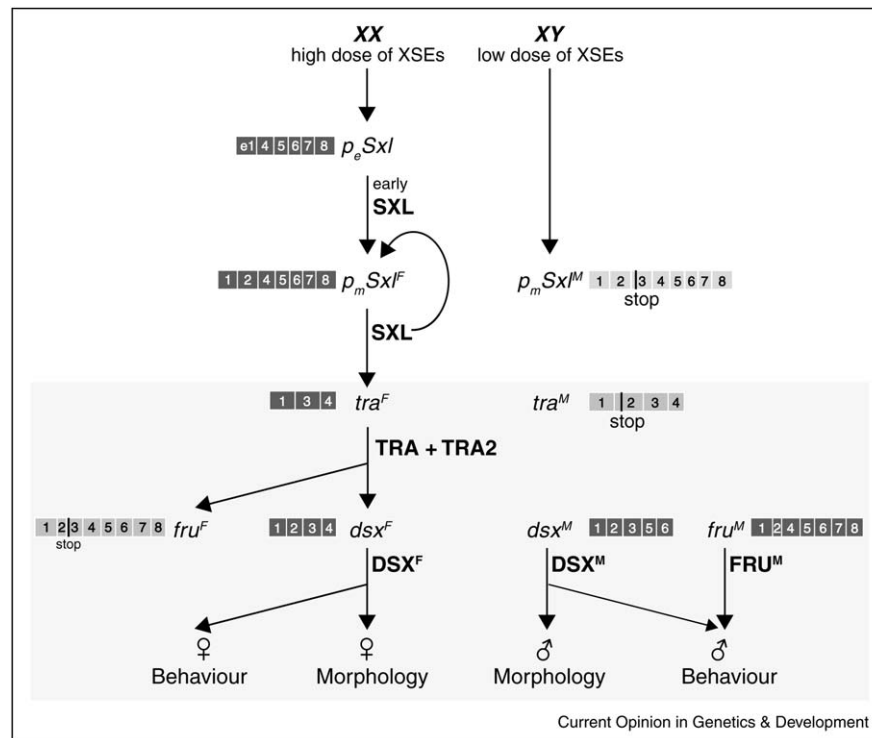
primary signals into one of two sex-specific pathways and consider how its function may serve as the key process around which insect sex determination mechanisms have evolved.

## Drosophila sex determination: the reference

Insect sex determination has been extensively examined in *Drosophila melanogaster* [4–6] and has served as a reference for all other insects [7–9]. In *Drosophila*, the upstream genomic region of *Sexlethal* (*Sxl*) contains two promoters:  $P_{\text{early}}$  and  $P_{\text{maintenance}}$ , which is the late promoter. The primary signal is based on the concentration of X-linked signal elements (XSE) that activate the early *Sxl* promoter in diploid XX individuals only [10•] (see Figure 1). Transcription from the early promoter of *Sxl* yields a transcript that is spliced to encode a functional early SXL protein. This splice pattern depends on the use of the 5' splice site from the early exon E1, whereas in later stages the 5' splice site of late exon 2 is used [11]. It results in the default exclusion of exon 3- that contains in-frame stop codons- in the early transcript. This early protein enables the production of a functional late SXL protein, which further maintains female-specific *Sxl* splicing by auto regulation. SXL also directs cryptic splicing of *tra* by binding to a polypyrimidine tract in the first *tra* intron and forces the general splicing factor U2AF to use the female-specific 3' splice site in exon 3 instead of the nonsex-specific 3' splice site in exon 2. This *tra* transcript yields a functional TRA protein [12–14], which interacts with the nonsex-specific transformer2 protein (TRA2) [15] and binds to the *dsx* transcript in the middle of exon 4, called the *dsx* repeat element (*dsxRE*). This *dsxRE* contains six copies of the 13 nucleotide sequence TC(T/A)(T/A)C(A/G)ATCAACA [16]. Located between repeat element five and six of the *dsxRE* is a purine-rich enhancer element (PRE) which is required for the specific binding of TRA2 to the *dsxRE* [17]. The binding of TRA/TRA2 to the *dsxRE* and PRE sites retains exon four in the *dsx* pre-mRNA resulting in female-specific splicing of *dsx* at the bottom of the cascade [18–20], generating a female-specific DSX protein.

In XY males the level of XSEs is insufficient for early *Sxl* transcription and no early SXL protein is synthesized, preventing the auto regulatory loop from establishing. As a result, *Sxl* pre-mRNA from the late promoter is male specifically spliced by default, yielding a truncated non-functional SXL protein. The absence of SXL leads to the 'default' splicing of the *tra* pre-mRNA and a nonfunctional TRA protein. Without TRA, *dsx* pre-mRNA is spliced by default generating a male-specific DSX protein.

Figure 1



The sex determination cascade in *Drosophila melanogaster*. Boxes with numbers indicate transcripts with exon number and relative exon size. Dark gray transcripts are full length and yield a functional protein. Light gray transcripts contain early in-frame stop codons and give truncated nonfunctional proteins. Transcripts are designated by their gene name in *italics*. Proteins are designated by their capital gene name. Superscript F and M stand for female-specific transcript or protein or male-specific transcript or protein, respectively. P<sub>e</sub>Sxl indicates Sxl transcript from the early promoter, P<sub>m</sub>Sxl indicates Sxl from the late promoter. DSX<sup>M</sup> directs primarily male morphology but also interacts a little with FRU<sup>M</sup> to direct male behavior, which is indicated by a smaller arrow. The gray bottom half of the sex-determining cascade shows the conserved part of the cascade.

The TRA/TRA2 complex also regulates female-specific splicing of *fruitless* (*fru*), which yields a nonfunctional FRU protein [21], while absence of TRA leads to male-specific *fru* splicing and a functional FRU protein. *Fru* is not part of the (morphological) sex determination pathway but seems conserved in insects [22,23] and reviewed in [24]. It is conserved in both gene structure and its function as a determiner of male sexual behavior.

### Conservation of sex-determining genes in insects

There is a common pattern in insect sex-determining cascades: at the bottom is *dsx*, which has been identified for all examined dipteran [25–32] and hymenopteran insect species [33,34]. DSX has two characteristic domains: a DNA binding domain (DM or OD1) and an oligomerization domain (*dsx* dimer or OD2). Oliveira *et al.* [34] showed for several insect species that amino acid alignment of these domains followed the established phylogeny, suggesting their importance in sexual differentiation. Conservation of *dsx* is in agreement with Wilkins' theory [35] stating that regulatory elements are recruited into sex-determining pathways, causing divergence towards the top, while *dsx* remains conserved at the

bottom. However, as more and more sex-determining cascades are elucidated, it appears that conservation is not only at the level of *dsx*, but also at the regulation of its sex-specific splicing.

### Transformer

After the initial identification in *Ceratitis capitata* [36], also in other insect species (*Anastrepha* sp., *Bactrocera oleae*, *Lucilia cuprina*, *Musca domestica*, *Apis mellifera* and *Nasonia vitripennis*), *dsx* splicing regulator genes have been identified that all appear to be *D. melanogaster tra* orthologs [37,38,39,40,41,42]. A *tra* ortholog has not (yet) been identified in Lepidoptera, perhaps because of the strong sequence divergence that characterizes *tra* evolution. In *Bombyx mori* no *tra* ortholog has been found based on the lack of *dsxRE* or PRE binding sites on *Bmdsx* and the presumed default mode of female-specific splicing [43,44]. However, *dsxRE*/PRE binding sites have only been identified in dipterans based on homology to *Drosophila* and are probably so diverged that recognition of these sites in other orders is difficult. Cho *et al.* [33] reported the absence of *dsxRE*/PRE binding sites in the hymenopteran *A. mellifera* and suggested that *Amdsx* follows default female-specific splicing, similar to *B. mori*.

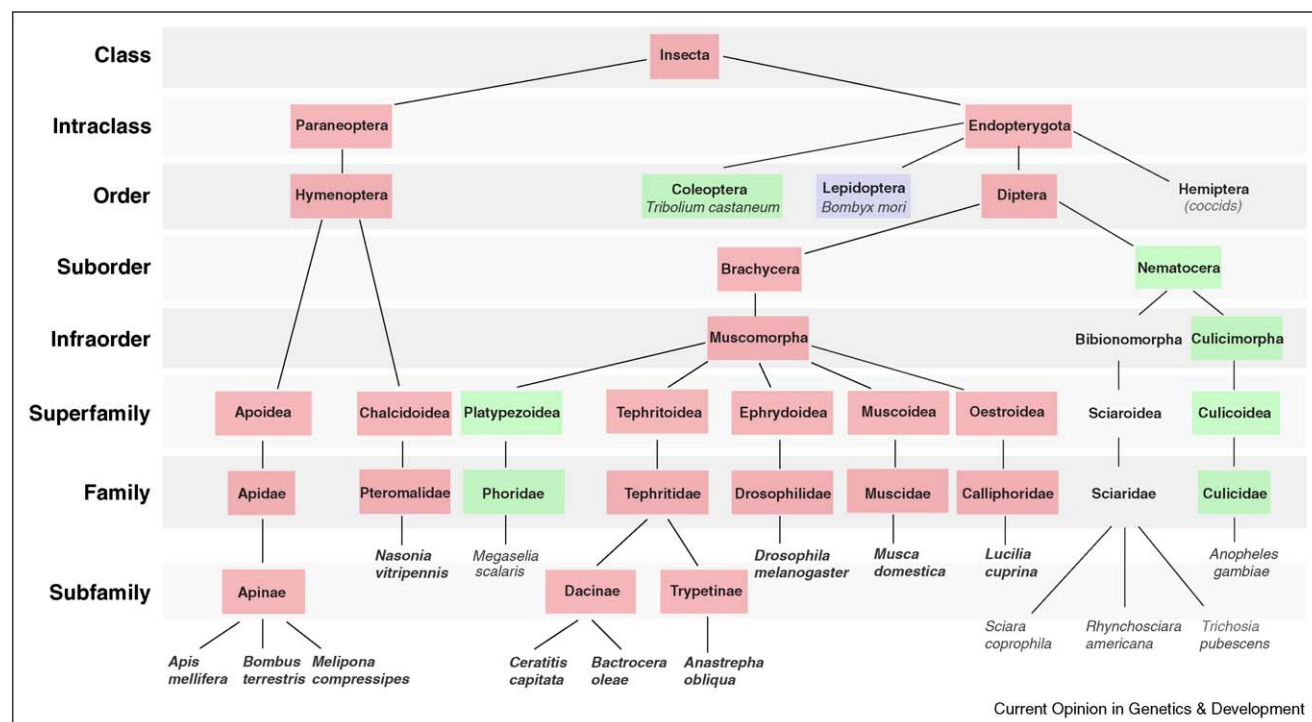
Nevertheless, a functional *tra* ortholog has recently been found in *A. mellifera*, termed *feminizer*, which is functionally and structurally similar to *tra* [39,45]. Interestingly, a comparison between *dsx* of hymenopterans *A. mellifera* and *N. vitripennis* revealed putative *dsx*RE/PRE binding sites that indeed have severely diverged from those of Diptera [23]. Similar *dsx*RE/PRE binding sites have been identified in the *Nasonia fruitless* gene [23] and in *Nvtra* [42]. Hence, the illustrious feminizing factor on the W chromosome in *B. mori* [46,47], may be an unconfirmed ortholog of *tra* that also functions as active feminizing factor. In the mosquito *Anopheles gambiae* and the phorid fly *Megaselia scalaris*, only *dsx* has been found to date, but *tra* is surmised to be the regulating splice factor of *Agdsx* and *Msdsx* since *dsx*RE/PRE binding sites have been identified [31,27].

The functional importance of these *dsx* splicing regulators in female development has been shown by RNA interference (RNAi) in early embryos, which resulted in male-specific *dsx* splicing [36,37,38,45,40,41,42]. The subsequent transformation of otherwise female offspring was not always complete and resulted in intersexes with various stages of masculinization, while male development remained unaffected. Although these *tra* genes differ largely in their nucleotide and amino acid composition, their function as the sex-specific splicing regulator

of *dsx* appears conserved [9]. Strikingly, a conserved pattern of *tra* regulation in all insect species is the sex-specific alternative splicing that produces transcripts in males that contain early in-frame stop codons and yield no protein. Only the female-specific splicing of *tra* pre-mRNA yields a full-length transcript and leads to TRA protein production. This active TRA protein directs female specific splicing of *dsx*, implying a functional conservation in insect sex determination.

*Tra* regulation of *dsx* apparently constitutes the axis of insect sex determination. It likely acquired its function in the early ancestors of the insects, as *tra* orthologs are found throughout the insect class including Diptera, Hymenoptera and Coleoptera (Figure 2), but apparently has no sex determining function in the crustacean *Daphnia* [48]. The large sequence divergence indicates that *tra* conservation is predominantly at the functional and less at the structural level. This becomes apparent when TRA protein sequences are compared among species. Comparison of the insect classification to a phylogeny based on the TRA protein sequence reveals that its evolution has followed species divergence confirming that conservation lies in function rather than sequence (Figure 3). Strikingly, alignment of TRA orthologs shows that only the proline and Arg/Ser rich regions are conserved throughout the examined insect species, reflecting their function as

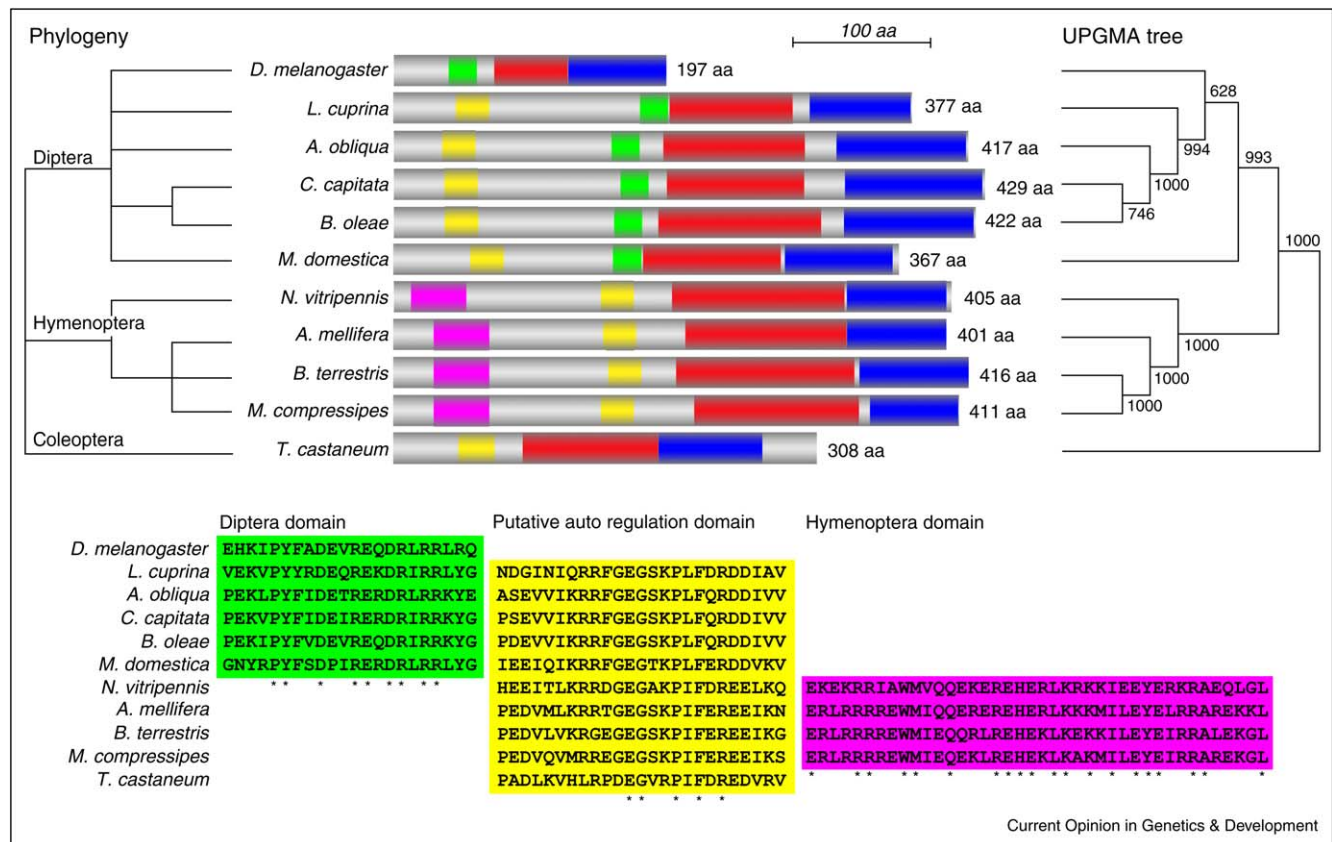
Figure 2



Classification of insects and the conservation of *dsx* and *tra*. Red boxes indicate species in which *dsx* and *tra* have been identified. Green boxes indicate species from which only *dsx* has been reported but presence of *tra* has been inferred from sequence data. Blue box indicates species in which a feminizing gene has been found but no *dsx* or *tra* yet (modified after [1]).



Figure 3



Amino acid alignment and protein sequence tree of *tra/fem* proteins identified in insect species from three different orders. Upper part: Insect classification on the left is redrawn from Sanchez [1\*\*]. On the right is the UPGMA consensus tree of the *transformer* protein sequences with bootstrap values indicated at the nodes (UPGMA cluster with a Jones–Taylor–Thornton matrix, 1000 bootstraps). In the middle is the protein sequence alignment showing all conserved areas. Green box indicates conserved domain in Diptera, yellow box indicates conserved domain in all species except *D. melanogaster* and purple box indicates conserved domain in Hymenoptera. Red box indicates shared Arg/Ser domains and blue box the common Pro-rich region. Lower part: Alignment of the conserved domains with similar colors as in the complete protein alignment (upper part). aa: amino acids. From top to bottom organisms and GenBank accession no.: *Drosophila melanogaster* (AAF49441); *Lucilia cuprina* (ACS34689); *Anastrepha obliqua* (ABW04165); *Ceratitis capitata* (AAM88673); *Bactrocera oleae* (CAG29243); *Musca domestica* (ACY40709); *Nasonia vitripennis* (NP\_001128299); *Apis mellifera* (ABV56235); *Bombus terrestris* (ABY74329); *Melipona compressipes* (ABV79891) and *Tribolium castaneum* (XP\_001809947).

splice factor. One additional domain is conserved in Hymenoptera only, a second domain is conserved in all species except *Drosophila* [48] and a third domain is conserved in all Diptera (Figure 3). The second domain may function in *tra* auto regulation that is absent in *D. melanogaster* and replaced by *Sxl*. The other two domains are apparently not involved in *tra* splicing but may have other unknown functions.

### Doublesex

*Dsx* belongs to a class of DM domain containing genes that are conserved outside the insect class, and regulates sex determination in both vertebrates [43,49–53] and invertebrates [54–56]. *Tra*, on the other hand, has been identified as a *dsx* splicing factor in insects only [48]. A comparison of sex-determining cascades in different insect groups reveals that diversity essentially starts at

the level of regulation of *tra* splicing and that *tra* acts as receptor for various primary signals. These signals are very diverse and include X-chromosome dose [10\*], a male determining factor on the Y chromosome and/or autosome [36\*\*,57], and a feminizing factor on the W chromosome [47] in diploids, as well as complementary sex determination and genomic imprinting sex determination in haplodiploids [42\*\*,58,59\*\*]. Therefore, the conserved part of the insect sex-determining cascade must be extended to include *tra*, and from an evolutionary perspective, *tra* appears to serve as the gene around which flexibility in sex determination is manifested.

### Evolution of *tra* regulation...

In all *tra* (or *fem*) containing insects except *D. melanogaster*, female specific splicing of *tra* involves an auto regulatory loop, in which the TRA protein is required for female

specific splicing of *tra* pre-mRNA [36<sup>••</sup>,60,40,38,45,41<sup>•</sup>,42<sup>••</sup>]. Maternal input of *tra* mRNA or protein into the eggs has been demonstrated for all examined species except *D. melanogaster* and *A. mellifera* and has been surmised to start *tra* auto regulation. In the haplodiploid *N. vitripennis* it has been shown for the first time that sufficient levels of maternally provided *tra* mRNA in eggs are required for female development. Knockdown of *tra* in mothers leads to a diminished amount of maternally provided *tra* mRNA in eggs and results in diploid males [42<sup>••</sup>]. In *Drosophila*, *Sxl* has been recruited upstream of *tra* and is female specifically regulated through its own auto regulatory loop. However, Siera and Cline [61<sup>•</sup>] showed that *tra* auto regulation may also be ancestral in *Drosophila* since a positive feedback loop of *tra* still operates through *Sxl*, which in turn regulates *tra* splicing. *Tra* regulation by X chromosome dose may occur outside the Drosophilidae but most likely in the absence of *Sxl*. How this is accomplished remains to be investigated. In *A. mellifera* a duplication of *fem* has been recruited into the sex-determining cascade and initiates female-specific splicing of *fem* transcripts [39<sup>•</sup>]. Overall, the maternal provision of *tra* to eggs appears to be an ancestral regulatory mechanism, as all deviations from this system are of recent origin.

Two intriguing questions are how variations in *tra* regulation can account for the large variety in sex determining mechanisms in insects and how turnovers in signals and genes controlling *tra* can occur during evolution. A comparison between diploid and haplodiploid sex determination is particularly illustrative as a 'flipover' of *tra* regulation may lie at the basis of the difference between these two modes of sex determination.

### ... in diploid insects

The principle of *tra* regulation in diploid insects is that the paternally inherited genome inhibits female splicing of *tra* in a variety of ways. A diverse array of primary signals directly or indirectly regulates sex-specific splicing of *tra*. A common theme in a number of dipteran insects is a masculinizing (M) factor on the Y chromosome that is transmitted through males only. M actively blocks the transcription or translation of *tra*, preventing the auto-regulatory loop from establishing in ways that are not yet well understood [36<sup>••</sup>,40,41<sup>•</sup>]. Thus, the paternally inherited M factor actively inhibits female development in XY individuals. In *Drosophila* the presence of twice as much X signal elements in XX animals directs female-specific transcription of *Sxl* and starts the female-specific path of the sex-determining cascade [10<sup>•</sup>].

A special case is Lepidoptera in which females are the heterogametic sex (ZW females, ZZ males) [46<sup>••</sup>]. As only females contribute a W chromosome containing a feminizing factor, males passively promote male development. In their theoretical treatise on the evolution of

sex determination, Pomiankowski *et al.* [62] inferred how, based upon initial allelic variation for *dsx* (*dsx*<sup>M</sup>: masculinizing factor and *dsx*<sup>F</sup>: feminizing factor) conversion to *tra* regulation can evolve. Assuming that TRA splices only *dsx*<sup>F</sup> into a female form but not *dsx*<sup>M</sup>, a mutation creating a stop codon in the *tra* exon 2 (*tra*<sup>S</sup>) would be favourable for *tra*<sup>S</sup>/*tra*<sup>S</sup> males, as no female DSX is produced. Simulations showed that this could eventually lead to elimination of *dsx*<sup>M</sup> and to evolution of female heterogamy for *tra*<sup>S</sup>/*tra*<sup>F</sup> [62].

Two general rules emerge from comparing the different primary signals of diploid insects. First, a paternally derived genome is always necessary for male development and second, actively or passively, it always prevents the activation of *tra* (or *Sxl*).

### ... in haplodiploid insects

A number of insect groups, including thrips (Thysanoptera), beetles (Coleoptera) and all Hymenoptera, have haplodiploid sex determination: males are haploid, develop from unfertilized eggs and only inherit a maternal genome, whereas females are diploid, develop from fertilized eggs and inherit a paternal and a maternal genome. It is therefore impossible for the paternally inherited genome to have a masculinizing effect as in diploids. Instead, the paternal genome must have acquired a complete reversal in sex determining function, that is by feminizing rather than masculinizing.

Until recently, knowledge about primary signals in haplodiploid species was limited to complementary sex determination (*csd*) in which gender is determined by the allelic state of the *complementary sex determiner* (*csd*) gene. Although *csd* has been inferred for more than 60 hymenopterans [63], the *csd* gene has been characterized in the honey bee only [58]. Females are heterozygous and males hemizygous at this locus, but the biochemical details of CSD function are not yet completely known [39<sup>•</sup>,45]. Interestingly, the *csd* primary signal can also be based on multiple loci (*ml-csd*) [64]. However, in all *csd* cases the paternally contributed genome provides the second *csd* allele that is required for female development.

In another hymenopteran, *Nasonia*, *csd* has been ruled out as the primary signal [65]. In *Nasonia* female-specific *tra* is maternally provided to eggs. In embryos from fertilized eggs early zygotic expression of *tra* is higher than in embryos from unfertilized eggs, which initiates an auto regulatory loop of *tra* and results in female *dsx* splicing [42<sup>••</sup>]. In embryos from unfertilized eggs no early zygotic expression of *tra* occurs and the auto regulatory loop does not establish, leading to male-specific *tra* and *dsx* splicing. The difference in zygotic *tra* expression cannot be explained by masculinizing factors. Instead, the *tra* gene, or a trans acting factor that influences *tra* expression, on the maternal genome is rendered inactive by maternal

imprinting. In an unfertilized egg, only this maternally imprinted gene is present that prevents *tra* transcription and precludes the auto regulatory loop. In a fertilized egg, both a maternal and a paternal genome are present. The paternal set has an active, nonsilenced gene so that *tra* will be transcribed enabling the maternally provided *tra* mRNA to start auto regulation of *tra*, eventually leading to female development [42<sup>••</sup>]. A mutant strain of *N. vitripennis* that produces gynandromorphs and females from haploid unfertilized eggs [66,67] may be explained by incomplete imprinting in the maternal germ line.

The honeybee and *Nasonia* results indicate that, in contrast to diploids, the paternally inherited genome is always necessary for female development in haplodiploids and, actively or passively, promotes the activation of *tra*.

## Conclusions and outlook

Much has been learned about sex determination in mammals [68] and plants [69–71], but comparative work on a variety of insect species has been particularly fruitful for understanding how sex determination regulation evolves. Twenty-five years ago Nöthiger and Steinmann-Zwicky [5] proposed that sex determination in all insects is based on a single principle. We can conclude that these authors were partly correct. The sex-specific regulation of *dsx* splicing by *tra* appears to constitute a conserved gene axis in all insects. Clearly, the central gene around which diversity evolves is not *Sxl*, as was suggested from studies in *Drosophila*, but *tra*. A striking example of the central role of *tra* in the evolution of insect sex determination is the complete reversal in the paternal regulation of *tra* upon the separation of Hymenoptera and Diptera.

Although *Drosophila* has XX–XY sex determination, its processing of the primary signal and regulation of *tra* is different from all other flies with this mode of sex determination. A number of other insect groups likely rely on sex chromosome dose as primary signal, such as species with XX–XO sex determination (e.g. grasshoppers (Orthoptera)), ZO–ZZ sex determination (e.g. some Lepidoptera [46<sup>••</sup>]) and paternal X chromosome inactivation (e.g. coccids (Homoptera) and Sciarid flies [1<sup>••</sup>]). Whether and how *tra* regulation occurs in these groups remains an interesting unanswered question. In general, a broader taxonomic screen of how primary signals are processed by *tra* would be worthwhile as our current knowledge is virtually restricted to Diptera and Hymenoptera. Exploiting next generation sequencing technology will greatly expedite such an endeavor.

## Acknowledgement

We thank Albert Kamping and Daniel Bopp for fruitful discussions on insect sex determination.

## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Sanchez L: **Sex-determining mechanisms in insects.** *Int J Dev Biol* 2008, **52**:837–856.
- Comprehensive review on sex determination mechanisms in insects covering five insect orders.
2. Saccone G, Pane A, Polito LC: **Sex determination in flies, fruitflies and butterflies.** *Genetica* 2002, **116**:15–23.
3. Dübendorfer A, Hediger M, Burghardt G, Bopp D: **Musca domestica, a window on the evolution of sex-determining mechanisms in insects.** *Int J Dev Biol* 2002, **46**:75–79.
4. Baker BS: **Sex in flies: the splice of life.** *Nature* 1989, **340**:521–524.
5. Nöthiger R, Steinmann-Zwicky M: **A single principle for sex determination in insects.** *Cold Spring Harbor Symp Quant Biol* 1985, **50**:615–621.
6. Schütt C, Nöthiger R: **Structure, function and evolution of sex-determining systems in Dipteran insects.** *Development* 2000, **127**:667–677.
7. Graham P, Penn JK, Schedl P: **Masters change, slaves remain.** *Bioessays* 2002, **25**:1–4.
8. Shearman DCA: **The evolution of sex determination systems in dipteran insects other than Drosophila.** *Genetica* 2002, **116**:25–43.
9. Pane A, De Simone A, Saccone G, Polito C: **Evolutionary conservation of Ceratitis capitata transformer gene function.** *Genetics* 2005, **171**:615–624.
10. Erickson JW, Quintero JJ: **Indirect effects of ploidy suggest X chromosome dose, not the X:A ratio, signals sex in Drosophila.** *PLoS Biol* 2007, **5**:e332.
- This work shows that the dose of only X-encoded signal elements is sufficient to start the sex-determining cascade in *D. melanogaster* instead of the X:A ratio and explains the sex of triploid intersexes and haploid individuals.
11. Zhu C, Urano J, Bell L: **The Sex-lethal early splicing pattern uses a default mechanism dependent on the alternative 5' splice sites.** *Mol Cell Biol* 1997, **17**:1674–1681.
12. Boggs RT, Gregor P, Idriss S, Belote JM, McKeown M: **Regulation of sexual differentiation in D. melanogaster via alternative splicing of RNA from the transformer gene.** *Cell* 1987, **50**:739–747.
13. Inoue K, Hoshijima K, Sakamoto H, Shimura Y: **Binding of the Drosophila Sex-lethal gene product to the alternative splice site of transformer primary transcript.** *Nature* 1990, **344**:461–463.
14. Valcarcel J, Singh R, Zamore PD, Green MR: **The protein Sex-lethal antagonizes the splicing factor U2AF to regulate alternative splicing of transformer pre-mRNA.** *Nature* 1993, **362**:171–175.
15. Amrein H, Gorman M, Nöthiger R: **The sex-determining gene tra-2 of Drosophila encodes a putative RNA binding protein.** *Cell* 1988, **55**:1025–1035.
16. Tian M, Maniatis T: **A splicing enhancer complex controls alternative splicing of doublesex pre-mRNA.** *Cell* 1993, **74**:105–114.
17. Lynch KW, Maniatis T: **Synergistic interactions between two distinct elements of a regulated splicing enhancer.** *Genes Dev* 1995, **9**:284–293.
18. Hoshijima K, Inoue K, Higuchi I, Sakamoto H, Shimura Y: **Control of doublesex alternative splicing by transformer and transformer-2 in Drosophila.** *Science* 1991, **252**:833–836.
19. Hedley ML, Maniatis T: **Sex-specific splicing and polyadenylation of dsx pre-mRNA requires a sequence that binds specifically to tra-2 protein in vitro.** *Cell* 1991, **65**:579–586.

20. Ryner LC, Baker BS: **Regulation of doublesex pre-mRNA processing occurs by 3'-splice site activation.** *Genes Dev* 1991, **5**:2071-2085.
  21. Ryner LC, Goodwin SF, Castrillon DH, Anand A, Vilella A, Baker BS, Hall JC, Taylor BJ, Wasserman SA: **Control of male sexual behavior and sexual orientation in *Drosophila* by the fruitless gene.** *Cell* 1996, **87**:1079-1089.
  22. Gailey DA, Billeter J-C, Liu JH, Bauzon F, Allendorfer JB, Goodwin SF: **Functional conservation of the fruitless male sex-determination gene across 250 Myr of insect evolution.** *Mol Biol Evol* 2006, **23**:633-643.
  23. Bertossa RC, van de Zande L, Beukeboom LW: **The fruitless gene in *Nasonia* displays complex sex-specific splicing and contains new zinc finger domains.** *Mol Biol Evol* 2009, **26**:1557-1569.
  24. Billeter JC, Rideout EJ, Dornan AJ, Goodwin SF: **Control of male sexual behavior in *Drosophila* by the sex determination pathway.** *Curr Biol* 2006, **16**:R766-R776.
  25. Shearman DCA, Frommer M: **The *Bactrocera tryoni* homologue of the *Drosophila melanogaster* sex-determination gene doublesex.** *Insect Mol Biol* 1998, **7**:355-366.
  26. Saccone G, Peluso I, Testa G, DiPaola F, Pane A, Polito C: ***Drosophila* sex-lethal and doublesex homologous genes in *Ceratitis capitata*: searching for sex-specific genes to develop a medfly transgenic sexing strain.** In *Proceedings of the Enhancement of the Sterile Insect Technique through Genetic Transformation using Nuclear Techniques*; IAEA/FAO: 1996:16-32.
  27. Kuhn S, Sievert V, Traut W: **The sex-determining gene doublesex in the fly *Megaselia scalaris*: conserved structure and sex-specific splicing.** *Genome* 2000, **43**:1011-1020.
  28. Hediger M, Burghardt G, Siegenthaler C, Buser N, Hilfiker-Kleiner D, Dübendorfer A, Bopp D: **Sex determination in *Drosophila melanogaster* and *Musca domestica* converges at the level of the terminal regulator doublesex.** *Dev Genes Evol* 2004, **214**:29-42.
  29. Lagos D, Ruiz MF, Sanchez L, Komitopoulou K: **Isolation and characterization of the *Bactrocera oleae* genes orthologous to the sex determining Sex-lethal and doublesex genes of *Drosophila melanogaster*.** *Gene* 2005, **348**:111-121.
  30. Ruiz MF, Stefani RN, Mascarenhas RO, Perondini ALP, Selivon D, Sánchez L: **The gene doublesex of the fruit fly *Anastrepha obliqua* (Diptera, Tephritidae).** *Genetics* 2005, **171**:849-854.
  31. Scali C, Catteruccia F, Li Q, Crisanti A: **Identification of sex-specific transcripts of the *Anopheles gambiae* doublesex gene.** *J Exp Biol* 2005, **208**:3701-3709.
  32. Ohbayashi F, Suzuki MG, Mita K, Okano K, Shimada T: **A homologue of the *Drosophila* doublesex gene is transcribed into sex-specific mRNA isoforms in the silkworm, *Bombyx mori*.** *Comp Biochem Phys B* 2001, **128**:145-158.
  33. Cho S, Huang ZY, Zhang J: **Sex-specific splicing of the honey bee doublesex gene reveals 300 million years of evolution at the bottom of the insect sex-determination pathway.** *Genetics* 2007, **177**:1733-1741.
  34. Oliveira DCSG, Werren JH, Verhulst EC, Giebel JD, Kamping A, Beukeboom LW, van de Zande L: **Identification and characterization of the doublesex gene of *Nasonia*.** *Insect Mol Biol* 2009, **18**:315-324.
- In this paper *doublesex* evolution is illustrated by domain peptide identity and serves well to be contrasted against our *transformer* evolutionary tree.
35. Wilkins AS: **Moving up the hierarchy: a hypothesis on the evolution of a genetic sex determination pathway.** *Bioessays* 1995, **17**:71-77.
  36. Pane A, Salvemini M, Bovi PD, Polito C, Saccone G: **The transformer gene in *Ceratitis capitata* provides a genetic basis for selecting and remembering the sexual fate.** *Development* 2002, **129**:3715-3725.
- In this paper it has been demonstrated for the first time that the *transformer* gene of *Ceratitis* is able to autoregulate, differently to the *Drosophila* orthologue. A transient depletion of maternal and zygotic *Ctra* mRNA by RNAi, causes complete masculinization of XX individuals and a permanent shift of the *Ctra* splicing into the male mode.
37. Ruiz MF, Milano A, Salvemini M, Eirín-López JM, Perondini ALP, Selivon D, Polito C, Saccone G, Sánchez L: **The gene transformer of *Anastrepha* fruit flies (Diptera, Tephritidae) and its evolution in insects.** *PLoS One* 2007, **2**:e1239.
  38. Lagos D, Koukidou M, Savakis C, Komitopoulou K: **The transformer gene in *Bactrocera oleae*: the genetic switch that determines its sex fate.** *Insect Mol Biol* 2007, **16**:221-230.
  39. Hasselmann M, Gempe T, Schiott M, Nunes-Silva CG, Otte M, Beye M: **Evidence for the evolutionary nascent of a novel sex determination pathway in honeybees.** *Nature* 2008, **454**:519-522.
- This study identified a *tra* homolog (*fem*) in honeybees and shows that *csd* is not an ancestral gene but arose from a duplication of *fem*.
40. Concha C, Scott MJ: **Sexual development in *Lucilia cuprina* (Diptera, Calliphoridae) is controlled by the transformer gene.** *Genetics* 2009, **182**:785-798.
  41. Hediger M, Henggeler C, Meier N, Perez R, Saccone G, Bopp D: **Molecular characterization of the key switch F provides a basis for understanding the rapid divergence of the sex-determining pathway in the housefly.** *Genetics* 2010, **184**:155-170.
- First study to show that the F factor in *M. domestica* is indeed *tra*, by carefully studying two mutant strains for F and relating findings to *tra* function.
42. Verhulst EC, Beukeboom LW, van de Zande L: **Maternal control of haplodiploid sex determination in the wasp *Nasonia*.** *Science* 2010, **328**:620-623.
- This study showed for the first time that imprinting may lie at the basis of sex determination in a hymenopteran without *csd*.
43. Suzuki MG, Ohbayashi F, Mita K, Shimada T: **The mechanism of sex-specific splicing at the doublesex gene is different between *Drosophila melanogaster* and *Bombyx mori*.** *Insect Biochem Mol Biol* 2001, **31**:1201-1211.
  44. Suzuki MG, Imanishi S, Dohmae N, Nishimura T, Shimada T, Matsumoto S: **Establishment of a novel in vivo sex-specific splicing assay system to identify a trans-acting factor that negatively regulates splicing of *Bombyx mori* dsx female exons.** *Mol Cell Biol* 2008, **28**:333-343.
  45. Gempe T, Hasselmann M, Schiott M, Hause G, Otte M, Beye M: **Sex determination in honeybees: two separate mechanisms induce and maintain the female pathway.** *PLoS Biol* 2009, **7**:e1000222.
  46. Traut W, Sahara K, Marec F: **Sex chromosomes and sex determination in Lepidoptera.** *Sexual Dev* 2007, **1**:332-346.
- Comprehensive review about sex determination in Lepidoptera and Trichoptera that share a female-heterogametic sex chromosome system.
47. Fujii T, Shimada T: **Sex determination in the silkworm. *Bombyx mori*: A female determinant on the W chromosome and the sex-determining gene cascade.** *Semin Cell Dev Biol* 2007, **18**:379-388.
  48. Kato Y, Kobayashi K, Oda S, Tatarazako N, Watanabe H, Iguchi T: **Sequence divergence and expression of a transformer gene in the branchiopod crustacean, *Daphnia magna*.** *Genomics* 2010, **95**:160-165.
  49. Cao J, Chen J, Wu T, Gan X, Luo Y: **Molecular cloning and sexually dimorphic expression of DMRT4 gene in *Oreochromis aureus*.** *Mol Biol Rep* 2009.
  50. Raghuveer K, Senthilkumaran B: **Identification of multiple dmrt1s in catfish: localization, dimorphic expression pattern, changes during testicular cycle and after methyltestosterone treatment.** *J Mol Endocrinol* 2009, **42**:437-448.
  51. Matsuda M, Shinomiya A, Kinoshita M, Suzuki A, Kobayashi T, Paul-Prasanth B, Lau E-I, Hamaguchi S, Sakaizumi M, Nagahama Y: **DMY gene induces male development in genetically female (XX) medaka fish.** *Proc Natl Acad Sci* 2007, **104**:3865-3870.
  52. Yoshimoto S, Okada E, Umemoto H, Tamura K, Uno Y, Nishida-Umehara C, Matsuda Y, Takamatsu N, Shiba T, Ito M: **A W-linked**



- DM-domain gene, DM-W, participates in primary ovary development in *Xenopus laevis*.** *Proc Natl Acad Sci* 2008, **105**:2469-2474.
53. Bratus A, Slota E: **DMRT1/Dmrt1, the sex determining or sex differentiating gene in Vertebrata.** *Folia Biol* 2006, **54**:81-86.
  54. Kato Y, Kobayashi K, Oda S, Colbourn JK, Tatarazako N, Watanabe H, Iguchi T: **Molecular cloning and sexually dimorphic expression of DM-domain genes in *Daphnia magna*.** *Genomics* 2008, **91**:94-101.
  55. Hodgkin J: **The remarkable ubiquity of DM domain factors as regulators of sexual phenotype: ancestry or aptitude?** *Genes Dev* 2002, **16**:2322-2326.
  56. Large EE, Mathies LD: **Chromatin regulation and sex determination in *Caenorhabditis elegans*.** *Trends Genet* 2007, **23**:314-317.
  57. Schmidt R, Hediger M, Roth S, Nothiger R, Dubendorfer A: **The Y-chromosomal and autosomal male-determining M factors of *Musca domestica* are equivalent.** *Genetics* 1997, **147**:271-280.
  58. Beye M, Hasselmann M, Fondrk MK, Page RE, Omholt SW: **The gene *csd* is the primary signal for sexual development in the honeybee and encodes an SR-type protein.** *Cell* 2003, **114**:419-429.
  59. Beukeboom LW, Kamping A, van de Zande L: **Sex determination in the haplodiploid wasp *Nasonia vitripennis* (Hymenoptera: Chalcidoidea): A critical consideration of models and evidence, Semin.** *Cell Dev Biol* 2007, **18**:371-378.
- Extensive review on sex determination in Hymenoptera without *csd*, testing all the described sex determining models against *Nasonia*.
60. Salvemini M, Robertson M, Aronson B, Atkinson P, Polito LC, Saccone G: ***Ceratitis capitata* transformer-2 gene is required to establish and maintain the autoregulation of *Cctra*, the master gene for female sex determination.** *Int J Dev Biol* 2009, **53**:109-120.
  61. Siera SG, Cline TW: **Sexual back talk with evolutionary implications: stimulation of the *Drosophila* sex-determination gene *Sex-lethal* by its target *transformer*.** *Genetics* 2008, **180**:1963-1981.
- First study to show a remnant of autoregulation at the level of *tra* in *Drosophila* and the evolution of the interaction between *sxl* and *tra*.
62. Pomiankowski A, Nothiger R, Wilkins A: **The evolution of the *Drosophila* sex-determination pathway.** *Genetics* 2004, **166**:1761-1773.
  63. Wilgenburg van E, Driessen G, Beukeboom LW: **Single locus complementary sex determination in Hymenoptera: an 'unintelligent' design?** *Frontiers Zool* 2006, **3**:1.
  64. de Boer JG, Ode PJ, Rendahl AK, Vet LEM, Whitfield JB, Heimpel GE: **Experimental support for multiple-locus complementary sex determination in the parasitoid *Cotesia vestalis*.** *Genetics* 2008, **180**:1525-1535.
  65. Werren JH, Richards S, Desjardins CA, Niehuis O, Gadau J, Colbourne JK, The Nasonia Genome Working Group: **Functional and evolutionary insights from the genomes of three parasitoid *Nasonia* species.** *Science* 2010, **327**:343-348.
  66. Beukeboom LW, Kamping A, Louter M, Pijnacker LP, Katju V, Ferree PM, Werren JH: **Haploid females in the parasitic wasp *Nasonia vitripennis*.** *Science* 2007, **315**:206.
  67. Kamping A, Katju V, Beukeboom LW, Werren JH: **Inheritance of gynandromorphism in the parasitic wasp *Nasonia vitripennis*.** *Genetics* 2007, **175**:1321-1333.
  68. Wallis M, Waters P, Graves J: **Sex determination in mammals — before and after the evolution of SRY.** *Cell Mol Life Sci* 2008, **65**:3182-3195.
  69. Tanurdzic M, Banks JA: **Sex-determining mechanisms in land plants.** *Plant Cell* 2004, **16**:S61-71.
  70. Martin A, Troadec C, Boualem A, Rajab M, Fernandez R, Morin H, Pitrat M, Dogimont C, Bendahmane A: **A transposon-induced epigenetic change leads to sex determination in melon.** *Nature* 2009, **461**:1135-1138.
  71. Ming R, Yu Q, Moore PH: **Sex determination in papaya.** *Semin Cell Dev Biol* 2007, **18**:401-408.